Fingolimod is an agonist of sphingosine-1-phosphate receptors and is used as a disease-modifying drug for multiple sclerosis (MS). We have previously shown that MS patients treated with fingolimod have reduced numbers of B cells in the circulation, and the remaining B cells in blood show an increased proportion of transitional B cells, that is, a naïve B-cell subset with anti-inflammatory properties. However, the mechanism of fingolimod-induced changes in the composition of circulating B cells is not yet clear. We hypothesized that B cell-activating factor of the tumor necrosis factor family (BAFF), which has critical roles in B cell maturation, selection, and survival, might underlie the alterations in B cell composition and the therapeutic effect of fingolimod in MS.

In this study, we analyzed serum concentrations of BAFF and associations with B cell subpopulations in MS patients treated with fingolimod. Furthermore, serum levels of soluble forms of TACI and BCMA, which are shown to reflect the activity of memory B cells and plasma cells in MS and SLE, were analyzed to gain insight into the effects of fingolimod on the systemic activity of these cell populations.

Methods

• Serum samples were obtained from 30 MS patients treated with fingolimod (MS-FGM), 32 patients without any disease-modifying drugs (MS-UT), and 25 healthy controls (HC). In addition, serum samples were obtained from 3 treatment-naïve MS patients before and 3 to 9 months after starting fingolimod treatment.

• Serum concentrations of BAFF, soluble (s) TACI and sBCMA were analyzed by ELISA.

• Monocytes isolated from blood of healthy donors were incubated with fingolimod-phosphate (100 ng/mL) or vehicle control for 24 h, followed by stimulation with IFNγ for 3 h. The expression levels of BAFF mRNA were quantified by qPCR.

• PBMC and B cell-depleted PBMC obtained from healthy donors were stimulated with Cpg ODN for 24 h, and the expression levels of BAFF mRNA were quantified by qPCR.

• The proportions of B cell subsets in 22 MS patients treated with fingolimod were determined by flow cytometry, and the relationships between serum BAFF concentrations and each B cell subset were analyzed.

Results

1. Increased serum BAFF concentrations in fingolimod-treated MS patients

2. No effect of fingolimod on BAFF expression in monocytes in vitro.

3. B cell-depletion from PBMC enhances BAFF production

4. Associations of serum BAFF concentrations and circulating total B cells in fingolimod-treated MS patients

5. Associations of serum BAFF concentrations and circulating B cell subsets in fingolimod-treated MS patients

Discussion and Conclusion

This study showed that MS patients treated with fingolimod had significantly higher levels of BAFF in the circulation. However, our in vitro experiment suggested that fingolimod did not directly enhance the production of BAFF, but rather, the observed BAFF elevation in serum was suggested to be secondary to the reduction of circulating B cell numbers by fingolimod.

Our results suggest that the elevation of serum BAFF levels underlies the increase in circulating transitional B cells in fingolimod-treated MS patients. Therefore, the composition of circulating B cells in fingolimod-treated MS patients is determined by both sequestration of CCR7B cells into lymphoid tissues and BAFF-mediated expansion of transitional B cells.

Our study on STACI and sBCMA suggests that, despite an increase of circulating BAFF levels, fingolimod-treated MS patients do not harbor enhanced memory B cells or plasma cell activity, as occurs in the case of SLE. This unique effect of fingolimod on B cells provides an advantage for the treatment of MS in which activated memory B cells producing pro-inflammatory cytokines are the key players that aggravate inflammatory T cell and myeloid cell activities.

In summary, fingolimod-treated MS patients have elevated levels of BAFF in the circulation, which are associated with the expansion of transitional B cells. Despite elevated BAFF levels, MS patients treated with fingolimod do not show signs of memory B cell or plasma cell activation. These unique effects of fingolimod on B cells are favorable for the treatment of MS.

Disclosure

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